Maturation of Gonads of Oysters, *Crassostrea virginica*, of Different Geographical Areas Subjected to Relatively Low Temperatures[1]

BY

VICTOR L. LOOSANOFF

Pacific Marine Station, University of the Pacific, Dillon Beach, California 94929[2]
and
Bureau of Commercial Fisheries, Biological Laboratory, Milford, Connecticut 06460

(Plates 19 to 25)

INTRODUCTION

The question of physiological variants in natural populations of a species has interested biologists for a long time. The problem was well summarized by Prosser (1955) who stated that, regardless of the general interest, the variations in physiological characters of races, or subspecies, have rarely been studied systematically. This lack of research is unfortunate because a comparison of physiological adaptations and requirements of closely related forms should contribute much to the understanding of intraspecific relations. Prosser, nevertheless, named several criteria used in distinguishing subspecies, and indicated that the response to temperature was one to be considered. My article deals with several aspects of gonadal development of oysters, *Crassostrea virginica* (Gmelin, 1791), of widely separated geographical areas, subjected to several different, relatively low temperatures.

The existence of physiologically different groups within the general population of *Crassostrea virginica* was first suggested by Coe (1934) during his studies of alternation of sexuality of these oysters. Long before Coe's statement was made, however, practical biologists noticed certain differences among the oysters of different areas of the Atlantic Coast. For example, Wells (1925), who discussed this matter at some length, stated that an oyster planter of New England or New York would prefer oysters originating in certain locations: "Today a bushel of seed from the famous Bridgeport bed will command double the price of Delaware or Chesapeake seed" (p. 19). Several years later, in studying spawning of Long Island Sound oysters Loosanoff & Engle (1942) concluded that this population was not homogeneous in its spawning behavior but consisted of different groups, perhaps races or subspecies, some of which required a higher temperature than others for completing maturation of their gonads and initiation of spawning. This opinion was shared by Stauber (1947, 1950) who, using information available in the literature on spawning of oysters of different areas, came to the conclusion that there are probably several physiologically different races within the general population of *C. virginica* of our Atlantic Coast.

The question remained speculative until Loosanoff & Nomejko (1951) presented the first evidence on the difference in temperature requirements for gonad maturation in several groups of *Crassostrea virginica*. These authors, working with oysters that originated in Massachusetts, Connecticut, New Jersey, and Virginia, demonstrated that, even though all the mollusks were of the same species, the temperature requirements for gonad development and successful spawning of the northern groups were lower than for groups living in warmer, southern waters. Preliminary examination of data based on new and much more extensive studies, in which samples again represented populations of different areas of our oyster producing belt from the Gulf of Mexico to Cape Cod were used, supported the original conclusion (Loosanoff, 1958a). Moreover, it is now generally ac-

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[2] Present address: 17 Los Cerros Drive, Greenbrae, California 94904, U. S. A.

ccpted that there are physiological subspecies in *Ostrea edulis* Linnaeus, 1758 (Korringa, 1957) and *Crassostrea gigas* (Thunberg, 1793) (Imai & Sakai, 1961) which differ in their responses to environmental factors. Recently, Hillman (1964) working with two groups of oysters, one indigenous to Long Island Sound and the other to James River, Virginia, offered additional evidence, based on chromatographic studies of intraspecific serological differences in *C. virginica*. A year later the same author (Hillman, 1965), using oysters representative of Long Island Sound, Delaware Bay, several sections of Chesapeake Bay, Virginia, and Louisiana, further demonstrated inherent metabolic differences among the populations.

MATERIALS AND METHODS

This article is based principally on the results of experiments performed and material collected while I was at the Bureau of Commercial Fisheries Laboratory, Milford, Connecticut. Additional material has been collected, however, and practically all microscopic examination and study of about 1500 histological preparations of gonads of oysters, as well as the analysis of the data, have been completed more recently, since I became associated with the University of the Pacific Marine Biological Station at Dillon Beach, California.

Some of the oysters used in these studies came from Long Island Sound. Others were shipped from New Jersey, Virginia, North Carolina, and Florida. All oysters were mature and were 3 to 6 inches long. Except for the lot from Florida (which was sent on November 5), they were shipped to Milford during the second half of September or early in October after, or near the end of, the spawning period.

The sources of the different lots of oysters were as follows: Long Island Sound, dredged in Milford Bay at a depth of about 30 feet; Miah Maull, New Jersey; the lower part of York River, Virginia (where the salinity is about 21 ppt); the vicinity of the State Laboratory near Bears Bluff, South Carolina; and Apalachicola Bay, Florida.

Upon arrival the oysters were suspended in wire trays in Milford Harbor to recover from shipment and to complete the extremely complex physiological processes taking place in their bodies at the end of the spawning period. These processes include the resorption of gonads and accumulation of glycogen and other reserve materials before the oysters begin hibernation (Loosanoff, 1942). They remained in the Harbor until the middle of January and then were transferred to the laboratory and placed in trays through which sea water of different temperatures was running at a constant rate.

Approximately 48 hours after the oysters were placed in Milford Harbor samples of gonads of all groups were taken for histological studies. All samples came from the same anatomical portion of the oyster, namely, the right side behind the line passing through the stomach on a level with the lower edge of the palps. In this way anatomical uniformity of the samples was assured. The blocks of gonadal tissue were carefully removed with a sharp razor blade to avoid pressure that could distort the tissue and produce various artifacts. The tissue was preserved in Bouin's solution and later processed by standard histological methods, sectioned at 5μ, and stained with iron-hematoxylin and eosin.

REACTIONS OF OYSTERS TO DIFFERENT TEMPERATURES

PRELIMINARY CONDITIONING AND EXPERIMENTAL ARRANGEMENTS

Examination of the Long Island Sound oysters, made approximately 48 hours after they were placed in Milford Harbor, showed that they were nearing completion of, or had completed spawning, and many had virtually undifferentiated gonads. In this group, resorption, which follows spawning, had been completed and the oysters were accumulating glycogen and other reserve materials (Plate 19, Figure 1). A few individuals, nevertheless, still contained small quantities of spawn. Gross examination showed that the meats were healthy and all oysters had crystalline styles and food in their stomachs.

The New Jersey oysters were not as far advanced as the Long Island Sound group; they had more individuals in the late stages of gonad resorption (Plate 19, Figure 2). The majority of these oysters, nevertheless, had already completed resorption and were approaching winter condition. All oysters contained large quantities of glycogen, and crystalline styles and large quantities of food were found in their stomachs.

The Virginia oysters were, as a rule, in the final stages of gonad resorption or already displayed winter-like gonads, although a few individuals of both sexes still contained different quantities of undischarged sexual products. Many oysters contained large quantities of glycogen (Plate 19, Figure 3). This observation suggests that a lack of this material could not be the reason for the failure of Virginia oysters to develop gonads later in the season when they were subjected to the conditioning

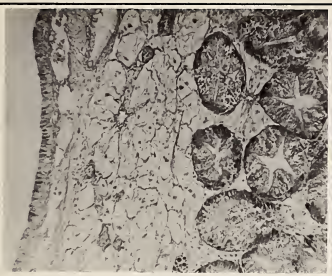
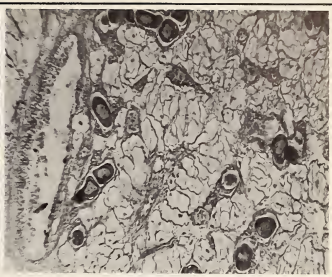


Figure 1



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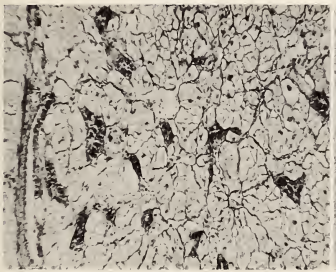

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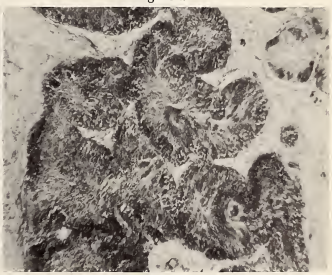


Figure 4

Figure 1: Gonad of a typical Long Island Sound oyster late in September after completion of spawning. The thin gonadal layer is confined between the body wall of the oyster and the tubules of the digestive diverticula. The small, undifferentiated gonadal follicles are surrounded by large cells of connective tissue containing glycogen. Later in the season, when the oysters have accumulated more reserve material, the glycogen-gonadal layer will become much thicker. (× 125)

Figure 2: Gonad of New Jersey female oyster late in September still containing some eggs that will soon be either discharged or resorbed. (× 125)

Figure 3: Gonad of Virginia oyster late in September. The small, newly formed gonadal follicles are surrounded by large masses of connective tissue. (× 125)

Figure 4: Gonad of South Carolina male oyster late in September still containing a large quantity of undischarged spermatozoa. (× 125)

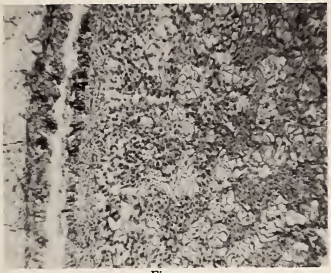


Figure 5

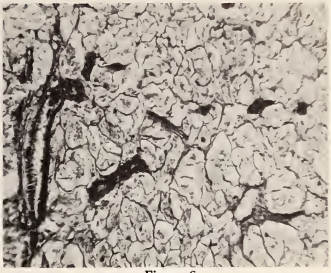


Figure 6

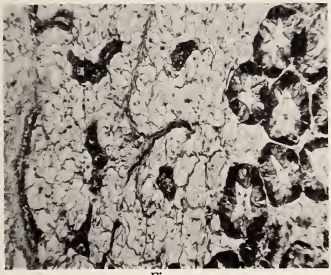


Figure 7

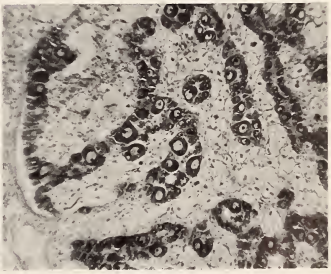


Figure 8

Figure 5: Gonad of Florida oyster collected on November 15. Gonadal follicles are virtually absent and a large portion of the entire area is occupied by leucocytes. Part of genital duct is visible. (× 125)

Figure 6: Gonad of Virginia oyster collected on January 15 containing small, undifferentiated follicles and large quantities of glycogen-laden tissue. (× 125)

Figure 7: Gonad of South Carolina oyster collected on January 15. (× 125)

Figure 8: Gonad of one of the most advanced Long Island Sound female oysters conditioned for 45 days at 12° C. (× 125)

methods employed at Milford Laboratory (Loosanoff, 1945; Loosanoff & Davis, 1950).

The South Carolina group was distinctly different from the 3 others described. Nearly all oysters still contained large quantities of spawn, and some appeared entirely unspawned (Plate 19, Figure 4). All were feeding well; their stomachs contained large quantities of food and all had large, well developed crystalline styles.

The first examination of the Florida oysters showed that they were in a much poorer condition than any of the previously described groups. They were "watery", and contained little glycogen or gonadal tissue (Plate 20, Figure 5). They had almost no gonadal follicles and the area between the digestive diverticula and the body wall was often filled with leucocytes or phagocytic cells. The presence of large numbers of these cells was characteristic of this group of oysters. Regardless of their poor condition, these oysters were feeding, although their crystalline styles were thin and their stomachs usually contained much less food than was found in the oysters of the other groups.

All groups remained suspended in Milford Harbor until January 15, at which time the bottom temperature was 3° C. Then, the oysters were brought into the laboratory, cleaned, and placed in trays of running water the temperature of which was about 5° C. The next day the temperature was increased to about 8° C and the oysters began to receive, as supplementary food, a mixture of phytoplankton grown in a tank of about 3000 liter capacity (Loosanoff & Engle, 1942 b).

All groups of oysters brought into the laboratory on January 15 were examined to ascertain their pre-experimental physical condition. The Long Island Sound oysters were in excellent condition, containing large quantities of glycogen; their gonads were in typical winter stage. The New Jersey oysters were in the same condition but contained even larger quantities of glycogen. The Virginia oysters were also in typical winter stage with undifferentiated gonadal follicles. None carried unspawned cells and, as a rule, all individuals had sufficient glycogen for normal development of gonads (Plate 20, Figure 6).

Although the South Carolina oysters still contained much spawn in September, by January 15 it was either entirely discharged or (more probably) resorbed in all individuals. Therefore, at the beginning of the conditioning experiments these oysters were in about the same physiological state as those of the 3 northern groups, since these South Carolina oysters also contained sufficient quantities of glycogen for development of gonads (Plate 20, Figure 7).

The Florida oysters differed radically from all other groups because, with the exception of a few individuals whose glycogen reserve was probably adequate (although considerably less than that of the other groups), the majority displayed a very thin glycogen-gonadal layer in which a few winter-like follicles were imbedded. Large numbers of blood cells were present throughout the bodies of these oysters, much the same as is shown in Figure 5, Plate 20. No individuals were found, however, with unspawned eggs or spermatozoa.

The general plan of the experiments was to hold some oysters of each geographic group at temperatures of 12°, 15°, 18°, 21°, and 24° C ± 1° C for different periods to determine the number of days required for maturation of their gonads, including the stage when the oysters become so ripe that they can be induced to spawn (Loosanoff, 1945; Loosanoff & Davis, 1963). In all trays the water temperature was increased gradually to the desired level to avoid possible physiological shock to the oysters. Positions of the trays were randomized and all trays received the same quantity of water and plankton food. Enough oysters were left in reserve for repetition of the experiments, if necessary, or for studies of the behavior of the oysters at higher temperatures. The present article discusses the observations made at comparatively low temperatures, namely, 12°, 15°, and 18° C ± 1° C.

In evaluating the condition of the oysters of the different groups, especially those of Florida, it was anticipated that many might be heavily infested with parasites, such as *Bucephalus*, or disease-causing forms, such as *Dermocystidium*. Because these organisms may adversely affect gonadal development, it was decided to base final conclusions only on those oysters which, upon microscopic examination, showed no easily recognizable parasites. It was realized, nevertheless, that even this precaution to achieve a fair approach in estimating the gonadal development was not entirely reliable because the numerous microorganisms that may affect oysters are not fully known. Nevertheless, the decision to base conclusions on only those oysters that appeared healthy helped, undoubtedly, to estimate more fairly the condition of the experimental animals.

Two criteria were used to evaluate the rate of gonad development. The first consisted in determining the number of days required, at a given temperature, for 50% of the oysters in the sample to develop physiologically ripe spermatozoa or fertilizable eggs. Attainment of this state was ascertained by examination of gonadal material from each oyster as it was dissected. This material was suspended in a small quantity of sea water and examined

under a microscope to determine whether it contained eggs or sperm. Gametes of the opposite sex were then added to determine if those of the examined oyster were physiologically ripe. The sex cells for this test were always taken from the oysters conditioned at a temperature of about 24° C and known to be entirely ripe.

The second criterion, applied chiefly to higher temperatures not discussed in this article, was to determine the length of the conditioning period necessary at each temperature before 50% of the oysters in a sample could be induced to spawn. This test was accomplished by placing each oyster in an individual container and then stimulating it to spawn by a rapid increase of water temperature to about 28° C or 30° C, and by simultaneous addition of a suspension of gonadal material taken from ripe oysters. Normally, each sample, especially those taken for final examination, consisted of 50 individuals. These groups will be discussed in geographic order, the northern oysters being considered first.

OBSERVATIONS AT 12° C

On March 4, after 45 days of conditioning at 12° C, the Long Island Sound oysters were again measured and examined. All showed new growth and some, whose total length was near 100 mm at the beginning of the experiment, had grown as much as 10 mm. The meats were good and the thickness of the gonadal layer varied from 0.5 mm to 1.0 mm. All stomachs contained food and crystalline styles were present.

None of the oysters of this group spawned when subjected to thermal and chemical stimulation. A later histological examination of the gonadal tissue showed that none were ripe and that many still had typical winter gonads characterized by small follicles containing virtually undifferentiated cells. Nevertheless, some advanced females possessed ovocytes measuring up to 30μ, although most of the cells in the follicles were considerably smaller (Plate 20, Figure 8). After a total of 50 days of conditioning at 12° C a larger number of females had ovocytes measuring between 25μ and 30μ, and in some males gametogenesis had progressed to the stage of formation of spermatids or, possibly, even a few spermatozoa. Even though the thickness of the gonadal layer of the most developed oysters was 1.5 mm, none could be induced to spawn.

The next group of Long Island oysters was examined after being kept for 61 days at 12° . They were subjected to spawning stimuli, by slowly raising the temperature of the water and holding it near 30° C for 2 hours, and by the addition of sperm and egg suspensions. None spawned. Histological examination of the gonad showed that the oysters varied greatly in degree of ripeness. Some females contained many eggs measuring 45μ, but the majority had unripe gonads in which the largest ovocytes were only about 15μ in diameter. Spermatozoa were found in several males (Plate 21, Figure 9). None of the ripe eggs of the normal females, to which sperm of the 12° C males was added, were fertilized, however, even though the sperm remained active for at least 2 hours. It was also noted that most of the spermatozoa suspended in the water were still connected by the heads in groups of 2 or 4, probably because they were not entirely ripe. Some of the eggs taken from the most advanced females were fertilized by sperm that came from males conditioned at a temperature of 24° C. Fertilization occurred but none of the eggs developed into straight-hinge larvae.

The first spawning of the male oysters conditioned at a temperature of 12° C was induced after 68 days. Spawning began when the temperature in the spawning dish reached 28° C. Discharged spermatozoa were apparently normal because they fertilized the eggs of females conditioned at higher temperatures; the eggs developed into normal straight-hinged larvae. The male spawned for 22 minutes, discharging a large quantity of spermatozoa.

A female was induced to spawn after 78 days. Initially, I attempted to induce spawning in this group merely by adding sperm and egg suspensions to the 12° C water in which the oysters were conditioned. When none of the oysters responded after 1 hour, the temperature was rapidly increased; when it reached 23° C, 15 minutes later, the first female responded. It was the only female that spawned in the group of 49 oysters. The eggs released were fertilized with normal sperm. Many embryos developed into abnormal larvae, which never progressed beyond the trochophore, but some reached the normal straight-hinge stage. Thus, experimental evidence was obtained that certain Long Island Sound oysters can be conditioned to ripeness at a temperature as low as 12° C.

Histological examination of the gonads of the oysters used in the spawning experiment after 78 days of conditioning showed that about 80% contained either large numbers of apparently normal spermatozoa or some fertilizable eggs (Plate 21, Figure 10) in contrast to the smaller groups that still had essentially winter gonads (Plate 21, Figure 11). This difference may indicate, as already suggested by Loosanoff & Engle (1942a), that the population of Long Island Sound is not genetically homogeneous.

The New Jersey oysters and the more southern groups were kept under the same experimental conditions as

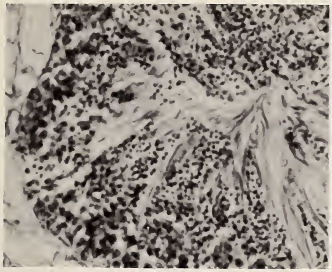
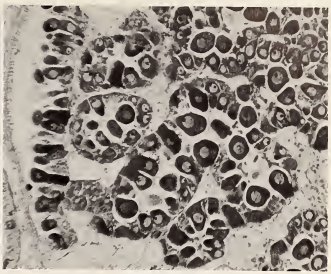


Figure 9



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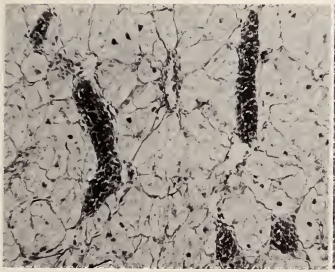

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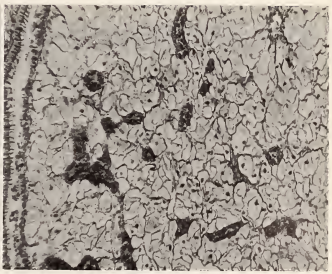


Figure 12

Figure 9: Gonadal follicle of Long Island Sound male oyster kept for 61 days at 12° C. The follicle contains ripe or nearly ripe spermatozoa. (× 575)

Figure 10: Gonad of Long Island Sound female oyster conditioned for 78 days at 12° C. This individual, one of the most advanced of the group, contains some ripe eggs. (× 125)

Figure 11: Gonad of Long Island Sound oyster showing almost no development after conditioning for 78 days at 12° C. The small follicles, just beginning to differentiate, are surrounded by large quantities of connective tissue. (× 125)

Figure 12: Gonad of one of the most advanced New Jersey oysters conditioned at 12° C for 67 days. Note small, undeveloped follicles and large quantities of connective tissue. Genital duct is visible. (× 125)

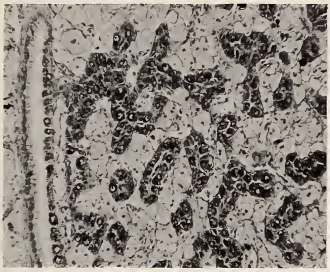
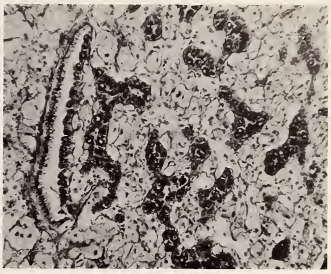


Figure 13



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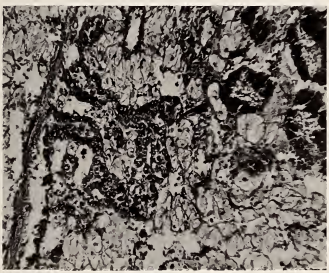

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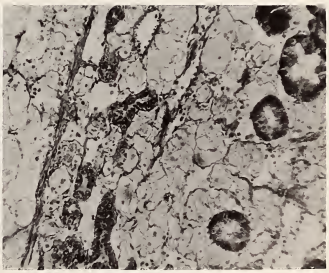

Figure 16

Figure 13: Gonad of New Jersey female oyster conditioned at 12° C for 78 days. (× 125)
Figure 14: Gonad of one of the most advanced Virginia oysters conditioned for 78 days at 12° C. (× 125)
Figure 15: Gonad of South Carolina oyster conditioned for 78 days at 12° C. (× 125)
Figure 16: Gonad of Florida oyster after 78 days of conditioning at 12° C. (× 125)